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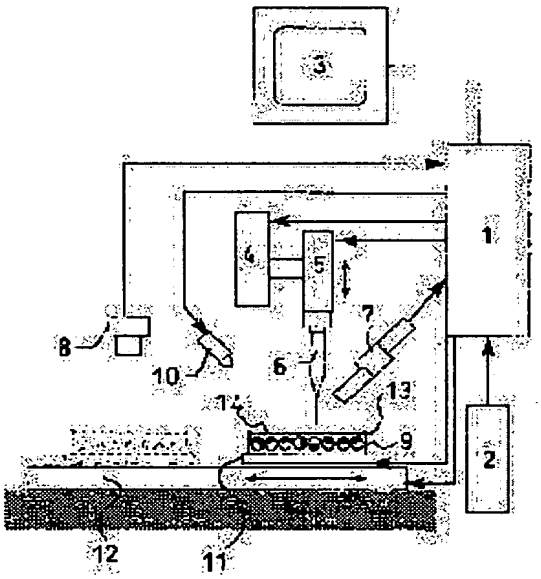
(54) APPARATUS FOR INTRODUCING SAMPLE INTOAMPHIBIAN OOCYTE

(57)Abstract:
PROBLEM TO BE SOLVED: To introduce a sample into amphibian oocytes at a prescribed depth with a good accuracy and retain the quality of the oocytes or a position where a needle is inserted as information.
SOLUTION: This apparatus for introducing a sample into amphibian oocytes is characterized as comprising a tray for holding plural amphibian oocytes, the introduction needle for introducing the sample into the plural amphibian oocytes, a driving part for moving the relative position of the tray and the introduction needle and a controlling part for inputting the depth of the introduction needle relatively to the tray or the amphibian oocytes during the introduction of the sample and introducing the sample into the plural amphibian oocytes at the depth.



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CLAIMS

[Claim(s)]
[Claim 1] The amphibian oocyte sample installation equipment carry out having the control section which make input the depth of the above-mentioned introductory needle to the above-mentioned tray or the above-mentioned amphibian oocyte of sample installation in the case as the mechanical component to which the relative position of the tray holding two or more amphibian oocytes, the introductory needle which introduce a sample into two or more above-mentioned amphibian oocytes, and the above-mentioned tray and the above-mentioned introductory needle moves, and controls the above-mentioned migration, and introducing a sample to the amphibian oocyte of the above-mentioned plurality in the above-mentioned depth as the description.
[Claim 2] The above-mentioned mechanical component is amphibian oocyte sample installation equipment according to claim 1 characterized by making the relative position of the above-mentioned tray and the above-mentioned introductory needle drive in the direction of a three dimension.
[Claim 3] Amphibian oocyte sample installation equipment according to claim 1 or 2 characterized by furthermore having the visual-information-acquisition section of the above-mentioned amphibian oocyte at the time of sample installation.
[Claim 4] The above-mentioned visual-information-acquisition section is amphibian oocyte sample installation equipment according to claim 3 characterized by being a camera.
[Claim 5] the amphibian oocyte sample installation equipment according to claim 3 or 4 which has further the means which relates with the location on the above-mentioned tray of each cell the vision information of each above-mentioned amphibian oocyte obtained by the above-mentioned visual-information-acquisition section.
[Claim 6] Amphibian oocyte sample installation equipment given in any 1 term of claims 3-5 characterized by having further the storage section which memorizes the above-mentioned vision information.
[Claim 7] The above-mentioned tray is amphibian oocyte sample installation equipment given in any 1 term of claims 1-6 characterized by having two or more holes for holding two or more above-mentioned amphibian oocytes.
[Claim 8] For a plane cylinder or a plane pars basilaris ossis occipitalis, a base is [the above-mentioned hole / a diameter at the maximum equator] amphibian oocyte sample installation equipment according to claim 7 to which it is characterized by being 1.4 to 2 mm in a cone form.
[Claim 9] For a plane cylinder or a plane pars basilaris ossis occipitalis, the base of the above-mentioned hole is amphibian oocyte sample installation equipment according to claim 7 with which it is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form.
[Claim 10] Amphibian oocyte sample installation equipment given in any 1 term of claims 1-9 characterized for the surface location of the oocyte on the above-mentioned tray by the thing of vision information, pressure variation, a temperature change, an electric change, humidity, and pH change which any one detects at least.
[Claim 11] The amphibian oocyte sample installation system carry out having the mechanical component to which the relative position of the tray holding two or more amphibian oocytes, the introductory needle which introduce a sample into two or more above-mentioned amphibian oocytes, and the above-mentioned tray and the above-mentioned introductory needle moves, the control section which control the above-

mentioned migration, the information acquisition section which acquire the vision information on the above-mentioned amphibian oocyte at the time of installation, and the storage section which accumulate the above-mentioned information, and introducing the above-mentioned sample to two or more above-mentioned amphibian oocytes as the description.

[Claim 12] The above-mentioned tray is an amphibian oocyte sample installation system according to claim 11 characterized by having two or more holes for holding two or more above-mentioned amphibian oocytes.

[Claim 13] For a plane cylinder or a plane pars basilaris ossis occipitalis, the base of the above-mentioned hole is the amphibian oocyte sample installation system according to claim 12 by which it is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form.

[Claim 14] The process which sets the depth of the above-mentioned tray or the above-mentioned introductory needle to the above-mentioned amphibian oocyte as the 1st depth using the equipment which has a tray holding two or more amphibian oocytes, and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes, The process which introduces a sample into the 1st oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle at the 1st depth of the above, The amphibian oocyte sample installation approach characterized by having the above-mentioned tray, the process to which the relative position of the above-mentioned introductory needle is moved automatically, and the process which introduces a sample into the 2nd oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle continuously at the 1st depth of the above.

[Claim 15] The process which introduces a sample into the 1st oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle using the equipment which has a tray holding two or more amphibian oocytes, and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes, The above-mentioned tray, the process to which the relative position of the above-mentioned introductory needle is moved, and the process which introduces a sample into the 2nd oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle continuously, The amphibian oocyte sample installation approach characterized by having the process which receives the condition of the oocyte at the time of the above-mentioned sample installation as vision information, and the process which accumulates the above-mentioned vision information.

[Claim 16] The above-mentioned sample is the amphibian oocyte sample installation approach according to claim 14 or 15 characterized by being a gene or protein.

[Claim 17] The above-mentioned sample is the amphibian oocyte sample installation approach given in any 1 term of claims 14-16 characterized by including a fluorescent material.

[Claim 18] The amphibian oocyte sample installation approach given in any 1 term of claims 14-17 characterized by controlling to make the same substantially the amount of installation of the sample introduced into the 1st oocyte of the above, and the sample introduced into the 2nd oocyte of the above.

[Claim 19] Two or more amphibian oocytes to which introductory depth of a sample is characterized by introducing the above-mentioned sample on equal conditions substantially, respectively.

[Claim 20] Amphibian oocyte according to claim 19 to which an introductory location is furthermore characterized by introducing the above-mentioned sample on the same conditions substantially.

[Claim 21] Two or more amphibian oocytes according to claim 19 or 20 characterized by the amount of installation of the above-mentioned sample being substantially more fixed still.

[Claim 22] The container characterized by having two or more amphibian oocytes to which introductory depth of a sample is characterized by introducing the above-mentioned sample on equal conditions substantially, respectively inside, and attaching the information about the time of the above-mentioned installation, and the information about the expiration date of the above-mentioned oocyte.

[Claim 23] The container according to claim 22 characterized by having the amphibian oocyte of further the above-mentioned plurality of the introductory location of the above-mentioned sample, or the amount of installation of the above-mentioned sample substantially introduced on equal conditions also about any one at least inside.

[Claim 24] Moving the relative location of the above-mentioned introductory needle to the above-

mentioned tray using the equipment which has a tray holding two or more amphibian oocytes, and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes Introduce a sample into each of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle, and the vision information of each amphibian oocyte on the above-mentioned plurality at the time of the above-mentioned installation comes to hand. The manufacture approach of amphibian oocyte that installation is characterized by collecting two or more oocytes introduced into **** of oocyte among two or more above-mentioned amphibian oocytes based on the above-mentioned vision information and that the sample was introduced.

[Claim 25] Moving the relative location of the above-mentioned introductory needle to the above-mentioned tray using the equipment which has a tray holding two or more amphibian oocytes, and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes Introduce a sample into each of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle, and the vision information of each amphibian oocyte on the above-mentioned plurality at the time of the above-mentioned installation comes to hand. The manufacture approach of amphibian oocyte that installation is characterized by collecting two or more oocytes introduced sober [oocyte] among two or more above-mentioned amphibian oocytes based on the above-mentioned vision information and that the sample was introduced.

[Claim 26] The amphibian oocyte group manufacture approach according to claim 24 or 25 which sets the depth of the above-mentioned amphibian oocyte or the above-mentioned introductory needle to the above-mentioned tray as the 1st depth before the above-mentioned installation, and is characterized by introducing a sample in the 1st depth of the above at two or more above-mentioned amphibian oocytes.

[Claim 27] The approach the introductory depth of a sample sells or transfers substantially two or more amphibian oocytes characterized by making a set two or more amphibian oocytes into which the sample was introduced, respectively on equal conditions.

[Claim 28] How to sell or transfer two or more amphibian oocytes according to claim 27 characterized by attaching the information about the sample installation to two or more above-mentioned amphibian oocytes to the above-mentioned set.

[Claim 29] The approach the introductory depth of a sample puts two or more amphibian oocytes introduced, respectively into a container, attaches the label which described the information about the sample installation to two or more above-mentioned amphibian oocytes in the above-mentioned container, and sells or transfers two or more amphibian oocytes on equal conditions substantially.

[Claim 30] The temperature of the above-mentioned container is the approach of selling or transferring two or more amphibian oocytes according to claim 29 by which it is characterized by being adjusted by 18 degrees C or more 22 degrees C or less.

[Claim 31] The information about the sample installation to two or more above-mentioned amphibian oocytes is the approach of selling or transferring two or more amphibian oocytes of a publication to any 1 term of claims 27-30 by which it is characterized by being the thing of the location which introduced the time of installation, the QA period of oocyte, and the sample, the depth which introduced the sample, and the probability of a manifestation concerning any one at least.

[Claim 32] The approach using two or more amphibian oocytes which introduced a gene or protein into almost fixed location and depth as a sensor for screening.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the automated equipment which uses the pipet's needle for the oocyte of amphibians, such as a frog, and introduces samples, such as a gene, coloring matter, protein, ** PUCHIDO, and a drug, into it. Moreover, it is related with the approach of selling or transferring the approach of introducing samples, such as a gene, coloring matter, protein, ** PUCHIDO, and a drug, into the specific location of amphibian oocyte, the amphibian oocyte quality was guaranteed to be about installation of a sample, and the amphibian oocyte which introduced the sample into a specific location and depth at the list.
[0002]
[Description of the Prior Art] The oocyte of frogs, such as a platanna which introduced samples, such as a gene, coloring matter, protein, ** PUCHIDO, and a drug Although it is widely used for the purpose, such as production of the protein as the check of an operation, the analysis of a gene function, and gene products, such as coloring matter to a viable cell, protein, ** PUCHIDO, and a drug, since size is obtained so much by low cost comparatively greatly Conventionally, each researcher bred the frog and was carrying out from extracting oocyte.
[0003] When introducing samples, such as a gene, coloring matter, protein, ** PUCHIDO, and a drug, into the oocyte of amphibians, such as a frog, conventionally, the engineer was stabbing manually the pipet filled up with these samples to oocyte under the microscope using the manipulator. An injector is equipped with a pipet and it carries out the regurgitation of the sample of a constant rate to intracellular with oil pressure or pneumatic pressure. Moreover, it considers as the approach of carrying out the seal of approval of the electrical potential difference, and carrying out the regurgitation of the sample of a constant rate to intracellular, and there are JP,5-192171,A and JP,6-343478,A. Any approach is the technique of bringing a needle close to a cell manually and introducing a sample, observing a cell under a microscope.
[0004]
[Problem(s) to be Solved by the Invention] Installation of the gene by the above-mentioned hand control etc. had the problem of changing the rate of the oocyte which has introduced the sample with an engineer individual's workmanship, the level of skill, etc. Since the numbers of oocytes which this can sample introduce per fixed time amount the whole engineer differ, it is one of the causes that whenever [conversion /-like at the time of ** of a sample] varies among engineers. Are concerned, there is no this invention in an engineer's workmanship and the level of skill, and it is setting to make regularity the number of processing per time amount to one of the purposes.
[0005] Moreover, the above-mentioned conventional technique was difficult to control depth also by the engineer who consideration to unification-izing sample installation depth to oocyte was not carried out, but became skillful. Consequently, it was to depend for the sample installation to specific intracellular organelles, such as a nucleus, by chance. This invention is setting to one of the purposes to make easy sample installation to the intracellular organelle in which control in the depth direction to a nucleus etc. is possible by unification-izing sample installation depth.
[0006] It was difficult not to carry out consideration to saving the information on the cell at the time of introducing a sample with the further above-mentioned conventional technique, but to acquire correlation with sample installation information and a subsequent cell reaction. Therefore, the purpose of this invention

is to acquire these correlation.
[0007] Moreover, since oocyte was supplied separately conventionally, mass production method and good production of the timing according to need were impossible. Therefore, other purposes of this invention are about the oocyte which introduced the specific sample, or the oocyte which guaranteed having introduced the specific sample into the fixed location to sell and convey [production and].
[0008]
[Means for Solving the Problem] This invention offers the equipment which uses the pipet's needle and introduces samples, such as a gene, coloring matter, protein, ** PUCHIDO, and a drug, into the location of the arbitration of the oocyte of amphibians, such as a frog, and the depth of arbitration in automatic control, in order to solve the above-mentioned technical problem.
[0009] Namely, the tray on which this invention holds two or more amphibian oocytes and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes, It has the control section which is made to input the depth of the above-mentioned introductory needle to the above-mentioned tray or the above-mentioned amphibian oocyte in the case of sample installation as the above-mentioned tray and the mechanical component to which the relative position of the above-mentioned introductory needle is moved, and controls the above-mentioned migration. The amphibian oocyte sample installation equipment characterized by introducing a sample into the amphibian oocyte of the above-mentioned plurality in the above-mentioned depth is offered.
[0010] Moreover, the tray on which this invention holds two or more amphibian oocytes and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes, The above-mentioned tray and the mechanical component which moves the relative position of the above-mentioned introductory needle in the direction of a three dimension, It has the control section which controls the above-mentioned migration, the information acquisition section which acquires the vision information on the above-mentioned amphibian oocyte at the time of installation, and the storage section which accumulates the above-mentioned information, and the amphibian oocyte sample installation system characterized by introducing the above-mentioned sample into two or more above-mentioned amphibian oocytes is offered. Thereby, it became possible to two or more amphibian oocytes to introduce a sample into almost fixed depth quickly.
[0011] Furthermore, as for the above-mentioned tray, a base has the hole where a plane cylinder or a plane pars basilaris ossis occipitalis is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form. The installation to the same field was attained about about 80 percent of two or more oocytes on a tray, without this using other special means.
[0012] this invention person etc. found out that proteinic functional manifestation effectiveness differed in the case where mRNA is sober introduced with the case where mRNA is introduced into **** of oocyte, when mRNA was introduced into oocyte. That is, in order to suppress dispersion in the functional manifestation effectiveness of the protein between oocytes, it becomes important to bring together the oocyte which introduced mRNA in the same field. In this invention, the information on the cell at the time of introducing a sample is saved, and it makes it possible to draw correlation with a subsequent cell reaction easily.
[0013] Furthermore, the equipment which has a tray holding two or more amphibian oocytes and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes is used for this invention. The process which sets the depth of the above-mentioned tray or the above-mentioned introductory needle to the above-mentioned amphibian oocyte as the 1st depth, The process which introduces a sample into the 1st oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle at the 1st depth of the above, The amphibian oocyte sample automatic installation approach characterized by having the above-mentioned tray, the process to which the relative position of the above-mentioned introductory needle is moved automatically, and the process which introduces a sample into the 2nd oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle continuously at the 1st depth of the above is offered.
[0014] The equipment which has a tray holding two or more amphibian oocytes and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes is used for this invention

further again. The process which introduces a sample into the 1st oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle, The above-mentioned tray, the process to which the relative position of the above-mentioned introductory needle is moved, and the process which introduces a sample into the 2nd oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle continuously, The amphibian oocyte sample automatic installation approach characterized by having the process which receives the condition of the oocyte at the time of the above-mentioned sample installation as vision information, and the process which accumulates the above-mentioned vision information is offered.

[0015] By invention of the above-mentioned amphibian oocyte sample installation equipment or an introductory approach, this invention offers two or more amphibian oocytes to which introductory depth of a sample is further characterized by introducing the above-mentioned sample on equal conditions substantially, respectively. Moreover, this invention offers the above-mentioned amphibian oocyte to which an introductory location is further characterized by introducing the above-mentioned sample on the same conditions substantially.

[0016] Furthermore, this invention offers the following. Moving the relative location of the above-mentioned introductory needle to the above-mentioned tray using the equipment which has a tray holding two or more amphibian oocytes, and the introductory needle which introduces a sample into two or more above-mentioned amphibian oocytes Introduce a sample into each of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle, and the vision information of each amphibian oocyte on the above-mentioned plurality at the time of the above-mentioned installation comes to hand. The manufacture approach of an amphibian oocyte group that installation is characterized by collecting two or more oocytes introduced into **** of oocyte, or two or more oocytes which were introduced sober [oocyte] among two or more above-mentioned amphibian oocytes based on the above-mentioned vision information and that the sample was introduced.

[0017] The approach the introductory depth of a sample sells or transfers substantially two or more amphibian oocytes characterized by for a sample making a set two or more amphibian oocytes introduced, respectively, and attaching the information about the sample installation to two or more above-mentioned amphibian oocytes on equal conditions.

[0018] Or the approach the introductory depth of a sample puts two or more amphibian oocytes introduced, respectively into a container, attaches the label which described the information about the sample installation to two or more above-mentioned amphibian oocytes in the above-mentioned container, and sells or transfers two or more amphibian oocytes on equal conditions substantially.

[0019] Here, the information about the sample installation to two or more above-mentioned amphibian oocytes is the thing about any one of the location which introduced the time of installation, the QA period of oocyte, and the sample, the depth which introduced the sample, and the probability of a manifestation at least. Therefore, it became possible to obtain two or more oocyte groups which make the conditions about installation the same substantially with those information by this invention.

[0020]

[Embodiment of the Invention] Below, this invention is explained further, referring to a drawing. The principle of this equipment is shown in drawing 1 . Although the tray on which 12 pieces opened horizontally the tray which carries out alignment arrangement of the oocyte, and the hole where a depth of a total of 96 pieces of eight pieces and a configuration are uniform opened it in the direct direction can be used, the number of holes of a tray is not necessarily limited to 96 pieces. Generally the sober weight of amphibian oocyte is large as compared with ****. Therefore, the oocyte which aligned on the tray is averaged without changing the sense by rotation etc., and the cell of about 80 percent turns **** up, is held, and it can raise the introductory probability to the same field by enlarging some from the oocyte using the bore diameter of a tray, without using a means special to others. The configuration of the hole for aligning oocyte is a flat surface where a base is circular, and is [the form of a cone form] desirable. [of a cylindrical shape with a fixed cross-section configuration parallel to the base from a base to opening or a pars basilaris ossis occipitalis] The diameter of opening of the above-mentioned hole needs to be more than a diameter of the amphibian oocyte to be used. Moreover, it confirms experimentally that it is desirable for there to be room which is extent which can rotate oocyte all over the above-mentioned hole

which filled the physiological saline etc. since upper mind was carried out, and it is desirable to make specifically used 105-150% of an oocyte diameter into the diameter at the maximum equator of a hole. For example, it becomes possible to fix in the specific direction, without damaging a cell by setting the diameter of a hole to about 1.4-2mm, since the diameter of the oocyte of a platanna is about 1.3mm. In this invention, the configuration of a suitable hole is the thing of a earthenware mortar mold with a depth [of the include angle of 90 degrees of a bottom as shown in drawing 2 , the diameter of 1.4mm, and a cylinder part] of 0.56mm. Moreover, the above-mentioned tray is used and also a syringe etc. is used, and the introductory probability to the same field can be gathered further, and it can also be operated so that it may turn up, specific field, for example, white side.

[0021] As a sample to introduce, a gene, coloring matter, protein, ** PUCHIDO, a drug, etc. are mentioned, and it is not limited especially. Moreover, although the following example indicates the oocyte which introduced Homo sapiens histamine receptor cRNA, the gene used for installation may not be limited to cRNA, and may be DNA and RNA, and the compound oligonucleotide. The needle for introducing a sample is not especially limited, although it is desirable that it is the pipet's needle. Moreover, although vision information through CCD camera 7 is made into the example as a means for detecting that the oocyte front face contacted [the introductory needle 6] the digital camera 8 in order to acquire cell information, such as sense of oocyte, a means required in order to acquire such information is limited to neither a digital camera 8 nor CCD camera 7. For example, cell surface is detectable based on such information by attaching the sensor which senses change of a pressure, temperature, the electrical and electric equipment, humidity, pH, etc. for introductory equipment.

[0022] After arranging the oocyte before sample installation in the hole of a tray 9 and filling the physiological saline 14 for amphibians on a tray 9, it installs on the direct movable carriage 11 and the horizontal migration base 12. Although it is desirable to determine the location of the oocyte 13 which introduces a sample from the introductory needle 6 by controlling migration of this direct movable carriage 11 and the horizontal migration base 12 by the control unit 1 to X shaft orientations and Y shaft orientations, a tray 9 can be fixed to the configuration and reverse of drawing 1, and the introductory needle 6 can also be considered as a movable configuration.

[0023] When a tray 9 is in the location of a broken line, information, such as a quality of oocyte and sense, can be accumulated by photoing oocyte with a digital camera 8 and sending the photography data to a control device 1.

[0024] The horizontal migration base 12 and the direct movable carriage 11 are operated with directions of a control unit 1, and the core of the first oocyte 13 which is in a position among the oocytes put in order by the tray 9 is moved to the lower part location of the transgenics needle 6. The introductory needle movable carriage 4 operates with directions of a control unit 1 for migration here of Z shaft orientations of an introductory needle, and it is made to descend to the location which left more slightly than the front face of oocyte the tip of the introductory needle 6 with which introductory equipment 5 was equipped, for example, several 100mm this side. Here, observing the image photoed with CCD camera 7 by the monitor 3, a command is taken out with the control auxiliary device 2, and the introductory needle movable carriage 4 is dropped at a low speed. It detects that the tip of the introductory needle 6 contacted the front face of oocyte 13 by vision information, pressure variation, the temperature change, an electric change, humidity, pH change, etc., and the introductory needle movable carriage 4 is stopped in this location. This location serves as a reference point of subsequent transgenics actuation. This location is stored in a control unit 1, and the following actuation is carried out. That is, the migration length of a vertical introductory needle and depth are set up to the field on which the tray to the location which introduces a sample from the above-mentioned reference point is put, the introductory needle 6 is stabbed with the depth by which a setup was carried out [above-mentioned], and the constant-rate regurgitation of the sample is carried out. For sample installation, Z five axis control of lowering an introductory needle to the oocyte 13 above-mentioned front face caudad 0.2mm from the location where the introductory needle 6 contacted can be performed. The optimal introductory depth of the introductory needle 6 to a cell changes with the classes of sample and the purposes of installation to introduce, and can be set up suitably. If it does not spread in a cell and is too deep when introductory depth of a sample is too shallow, the probability which damages a nucleus or hurts its cell will become high. Therefore, to installation of a sample, introducing into almost fixed depth is

desirable also from the point of manifestation effectiveness. For example, mRNA is introduced into intracytoplasmic and it is desirable to stab a needle in depth of 0.02-0.1mm from cell surface to discover protein. DNA is introduced in a nucleus and it is desirable to stab a needle in depth of 0.05-0.2mm from cell surface to discover protein on the other hand. However, since the configuration of oocyte is slightly distorted by contact of a needle at the time of installation, a sample is introduced into a location shallower than the depth set up in fact. The time amount for sample installation is controlled by setting up the time amount by which the needle is poured into intracellular according to the injection rate to a cell etc. In addition, in order to raise introductory effectiveness further, the introductory needle 6 can also be made into plurality. In this case, the mechanical component of equipment can also be made enough [moving the relative location of an introductory needle and a tray in-dimensional / 1 / or the two-dimensional direction].

[0025] Then, a sample is automatically introduced based on the three-dimension-positional information of the first oocyte with the time amount, the rate, and the introductory depth which were directed to the oocyte of the number of the arbitration after the second of a tray 9 by automatic control. Moreover, whenever it is installation, the function to perform surface location detection can also be made to have, since dispersion may be in the magnitude of oocyte. Moreover, the cell information on oocyte can be stored in a computer, and it can also be pulled out at the time of the need.

[0026] Furthermore, a motion of the above-mentioned introductory needle 6 at the time of sample installation and oocyte 13, the vision information on the oocyte at the time of installation, etc. can be stored in a computer, and it can also set up so that the description of a sample installation location, depth, and a cell etc. may be read after introductory actuation termination. In this case, as for the vision information about each oocyte, it is desirable by carrying out [attach / a number] to carry out by relating with the location on the above-mentioned tray.

[0027] Moreover, in the case where mRNA is introduced into the oocyte of an amphibian as sober sober [although it is known that there is **** and it was known that functions differ, respectively, when this invention person etc. introduces mRNA into oocyte] as the case where mRNA is introduced into **** of oocyte, it found out that proteinic functional manifestation effectiveness differed. That is, when introducing histamine receptor mRNA, manifestation effectiveness is higher than the case where it introduces sober [direction] having introduced into ****, and the good oocyte of ligand responsibility can be obtained. the direction sober introduced on the other hand when the protein containing a chromophore, fluorescence protein or these genes, and coloring matter were introduced -- the information on a color or light -- sensibility -- it can obtain highly. The installation to **** is [cell / on a tray] possible about about 80 percent by using the above-mentioned tray, as described above. Or as described above, each cell is operated using a syringe etc. to obtain the cell which introduced the sample into the specific field of a cell, for example, ****, and the sober chisel so that only a specific field may turn up beforehand before sample installation. Or in case a sample is introduced, the sample installation positional information of cell surface can be acquired by the vision information detection means or monochrome distinction sensor, and the cells in which the sample was introduced only into the cell from which the target field turned up can also be collected from the acquired information. The oocyte group which makes the conditions of an introductory location the same substantially by this is obtained. In this invention, "a specific location" means the location a sober side, near the equator, etc. the **** side of oocyte.

[0028] By using the equipment of the above-mentioned configuration, a sample can be introduced into the specific location and depth of amphibian oocyte, and the functional manifestation effectiveness (introductory effectiveness) of the introduced sample can produce the oocyte of this quality quickly and in large quantities mostly. Therefore, this invention also offers the approach of introducing a sample into the specific location and the depth of amphibian oocyte using the equipment of this invention.

[0029] By using the equipment of this invention showed that sample installation effectiveness improved as follows. That is, although it takes about about 30 minutes and introductory effectiveness is about 30% in the incidence rate at the time of making a gene into a sample in order to introduce a sample into 25 cells when it is the beginner who is inexperienced in the sample installation by manual operation, by use of the equipment of this invention, the time amount for introducing a sample into 25 cells becomes for only 3 minutes, and introductory effectiveness reaches to about 80%. Although such compaction is not looked at

by the time amount which it takes for installation in the case of the expert of the sample installation by manual operation, when it is 80% manually, introductory effectiveness can rise further by use of equipment, and can be attained even to 90%.

[0030] Therefore, without being dependent on an operator's workmanship by using the equipment and the approach of this invention, about 80 - 90% of introductory effectiveness was attained, and it became possible to sell or transfer two or more oocytes by which sample installation conditions were controlled by this invention for the first time. Therefore, in another viewpoint, this invention offers the amphibian oocyte installation of the sample to a specific location and depth was guaranteed to be.

[0031] Moreover, only the oocytes which introduced the sample into a specific location and depth can be collected, and it can sell or transfer. In the case of sale or transfer, packaging of two or more oocytes can be carried out, and the label 22 which indicated information, such as setups about the location and depth which introduced the class of sample, introductory time, the term of a guarantee of quality, and a sample, the introductory effectiveness guaranteed can be attached (drawing 3).

[0032] Furthermore, this invention can also attach the information about the proteinic manifestation in which the gene location [the gene] and introduced into the specific depth pan carries out a code, and can sell or transfer oocyte. In case the oocyte which guaranteed sample installation effectiveness and manifestation effectiveness is sold and transferred, cointroduction of the gene which carries out the code of the protein containing coloring matter or a chromophore, fluorescence protein, or such protein can be carried out to a sample, the number of oocytes which emits colored or fluorescence can be counted, and sample installation effectiveness can be guaranteed for the rate as an index of sample installation effectiveness. Although cointroduction may be performed with a mixed gestalt, when both the protein for the sample and detection which carry out cointroduction etc. is the gestalten of a gene, it can also consider as the gene which carries out the code of the fusion protein.

[0033] The example which calculates against an index the fluorescence from the fluorescence protein which carried out cointroduction of the introductory effectiveness or manifestation effectiveness of a sample is explained using drawing 4 . In this example, although the manifestation of OWAN jellyfish origin green fluorescence protein (GFP) is stated to an index about the example which authorized the introductory rate of a histamine receptor gene, this invention does not limit the sample to introduce to a gene. Moreover, the matter used as an index of the introductory effectiveness of a sample is not limited to GFP, either.

[0034] The mixture of a histamine receptor gene and a green fluorescence protein gene is introduced sober [oocyte] using equipment equipped with the above-mentioned device, or the above-mentioned principle. If light with a wavelength of 488nm is irradiated after transgenics actuation at the oocyte which passed for 24 hours, what green fluorescence protein discovered will emit 507nm fluorescence. ** is classified for the cell which emitted 507nm fluorescence, and a cell without **** is classified for fluorescence as dark. Furthermore, it is drawing 4 which carried out the histamine stimulus of these cells, and was classified according to the existence of a response. As shown in drawing 4 , in the cell of **, 85% of cell (inside of 40 pieces 34 pieces) answered the histamine. That is, the histamine receptor gene can be introduced into 85% or more of cell. Conversely, in the cell of dark, 90% or more of cell did not answer a histamine (inside of 28 pieces 27 pieces). That is, the histamine receptor gene cannot be introduced into 90% or more of cell. That is, it became clear that the oocyte which green fluorescence protein has discovered has the high rate that the histamine receptor gene is also introduced.

[0035] If cointroduction of the target sample is carried out to fluorescence protein etc. so that clearly from this, it is possible to be able to calculate the introductory effectiveness of the target sample by making existence, such as the fluorescence, into an index, to guarantee sample installation effectiveness, and to sell or transfer oocyte.

[0036] Next, although a means to produce the oocyte obtained by the approach of this invention for the specific application using the example using the oocyte which introduced Homo sapiens histamine receptor cRNA, and to sell or transfer is indicated, the gene used for installation may not be limited to cRNA, and may be DNA and RNA, and the compound oligonucleotide.

[0037] The equipment and the approach concerning above-mentioned this invention are used, and histamine receptor cRNA is introduced into oocyte. Under the present circumstances, if a means to acquire

vision information, such as monochrome distinction sensor, or a CCD camera/digital camera, is used, the oocyte which introduced cRNA into ****, and the oocyte which introduced cRNA sober can be classified.
[0038] A histamine receptor is discovered to oocyte within [in 24 hours] after transgenics actuation. After histamine receptor transgenics actuation, it passes for 24 hours or more, and the membrane potential of the oocyte considered that the histamine receptor was discovered is fixed to -60mV with a 2 electrode-layer voltage clamp method. If the sample which contained the histamine in oocyte under such a condition is added, since the histamine and histamine receptor in a sample will interact, the signal transduction system in oocyte will be activated and an ionic current will occur, oocyte shows an electric response to a histamine. The oocyte which passed through 24 hours is stimulated with 1microM histamine, and the current response is measured. It is drawing 5 which compared the magnitude of **** or a current response of the oocyte which introduced the gene sober. As shown in drawing 5 , the difference in the current response to a histamine stimulus was looked at by at least gene induction. That is, it was shown rather than the case where the direction at the time of introducing a gene into **** introduces a gene sober that oocyte with more sufficient ligand responsibility is obtained.
[0039] The oocyte ensemble who followed, for example, was introduced only into **** in histamine receptor cRNA can use it as a highly sensitive sensor to a histamine. Furthermore, the oocyte which introduced the sample into a specific location and depth can be sold or transferred for the purpose of using for a sensor by this invention. The antibody which has the reactivity over the gene of the acceptor over the ligand of arbitration other than a histamine and a specific antigen, the glycoprotein which has a specific sugar chain are mentioned, and the sample which can be introduced in order to use as a sensor the oocyte obtained by this invention for this contractor so that it may be recognized easily is not limited especially.
[0040] The histamine receptor gene 31 is introduced into oocyte 13 using equipment equipped with the above-mentioned device, or the above-mentioned principle. The case where it introduced into drawing 6 sober for convenience was indicated. A histamine receptor 32 is discovered to oocyte 13 within [in 24 hours] after histamine receptor transgenics actuation. Like the above, after histamine receptor transgenics actuation, it passes for 24 hours or more, and the membrane potential of the oocyte considered that the histamine receptor 32 was discovered is fixed to -60mV with a 2 electrode-layer voltage clamp method. If the sample 33 which contained the histamine in oocyte 13 under such a condition is added, since the histamine and histamine receptor in a sample will interact, the signal transduction system in oocyte will be activated and an ionic current will occur, oocyte 13 shows the electric response 34 to a histamine. Oocyte 13 is 36 which does not answer [as opposed to / since the matter which interacts with an acceptor does not exist when the sample 35 which does not contain a histamine is added / a histamine].
[0041] That is, a sample can be added to the oocyte which introduced the histamine receptor gene, existence of the cell response can be made into an index, and sensing of the existence of the histamine in a sample can be carried out. Therefore, it became possible to use amphibian oocyte for screening, such as ligand reacted to a certain acceptor and an antibody, or an antigen, by the ability of the oocyte which makes the introductory conditions of a sample the same to be produced now in large quantities by using the sample installation equipment which is this invention. Namely, a gene etc. can be substantially introduced under the same conditions and it can screen by comparing the result by making ligand which is different in two or more oocytes of each which made protein etc. discover react.
[0042] Moreover, although the protein discovered by carrying out [crush / as another application / the oocyte which has discovered protein etc.] can be extracted, it is possible to extract desired protein etc. efficiently by using the cell which controlled introductory conditions using the introductory equipment of this invention, for example, introduced the sample into ****.
[0043] Next, how to convey the oocyte which introduced the sample concerning this invention is indicated according to drawing 6 . In case the above-mentioned oocyte is sold and conveyed, as shown in the buffer solution usually used for the oocytes of an amphibian at drawing 3 , putting oocyte 13 into the container 21 which filled the solution which added antibiotics, such as gentamicin sulfate, penicillin, and streptomycin, using packaging, such as styrene foam, and avoiding an impact, 4-25 degrees C of temperature are kept desirable at 18-22 degrees C by use of a cold insulator 23 etc., and conveying is desirable.
[0044] Although especially the presentation of the above-mentioned solution is not limited, it can use the thing of the following presentations suitably, for example. pH of this solution is 7.5.

NaCl 96mMKCl(s) 2mMCa[illegible](s)2 1.8mMMgCl(s)2 1mMHEPES 5mM gentamicin sulfate 50microg [/ml] pyruvic-acid sodium 2.5mM penicillin 10U/ml streptomycin 10microg [0045]/ml It is desirable for a cell to be able to move comparatively freely within a container 21, not to be limited as a container suitable for sale and transportation, especially if sealing with the lid which can be opened and closed is possible, and to fill the above-mentioned solution of the volume 90 percent till about 5 minutes. For example, in the case of a 50ml conical tube, it is desirable about 100-200 oocytes and to put in about 130-180 cells preferably. Although this rate is equivalent to about about 0.3-0.5ml to one oocyte, it may change according to the class of oocyte, the class of container, etc.

[0046] As shown in drawing 7 , the oocytes 13 which introduced the sample are collected from a tray 9 using syringe 41 grade. Under the present circumstances, only **** or the oocyte into which the sample was introduced sober is also recoverable based on the information recorded previously. The collected oocyte is moved to a container 21. The buffer solution 42 for amphibians is filled in the container 21 concerned, and liquid is exchanged several times. After exchange, again, the buffer solution 42 for amphibians is filled in a container 21, and the antibiotic 43 of optimum dose is added further. The lid of a tube is shut and it puts into the outer container 44 containing a cold insulator. Packaging 45 grade is put into an outer container 44, a container 21 is fixed, and it carries to a purchase place with a general means of transportation.

[0047] It can carry to a purchase place, without spoiling the function of the oocyte which introduced the sample by this approach. Moreover, in the case of sale and transfer, information, such as introductory conditions, such as a location which introduced introductory time and a sample, and depth, recall, and a QA period, is offered together. As the approach, the space which indicated those information may be attached or a label 22 may be attached to the container 21 which has two or more oocytes (drawing 3).

[0048] The time which took about about 24 hours by the manifestation when a gene was introduced, and was introduced after installation as a life of oocyte since about 5 day room became a standard more preferably, about 7 day room and, and since the period after effective installation is specified corresponding to it, it is desirable to indicate "the use by the O moon O day is desirable" etc. About the oocyte of about 80 percent, the installation to **** is possible by using the above-mentioned tray about an introductory field, and it is also possible to raise the extent about manifestation effectiveness based on the information on the oocyte accumulated in the above-mentioned equipment using coexpression.

[0049]

[Effect of the Invention] By this invention, it can be accurate for the oocyte of amphibians, such as a frog, with fixed depth, a sample can be introduced, and introductory effectiveness etc. can obtain the oocyte of this quality quickly and in large quantities. The location where the quality of oocyte or the needle was inserted can be saved as information. Moreover, it is possible to collect only the oocytes which introduced the sample into the specific location and depth which were obtained by the approach of this invention, or to guarantee sample installation effectiveness, and to sell or transfer. Furthermore, the class of introduced sample is embraced, and an application can be specified, and it can also sell or transfer.

[Translation done.]

* NOTICES *

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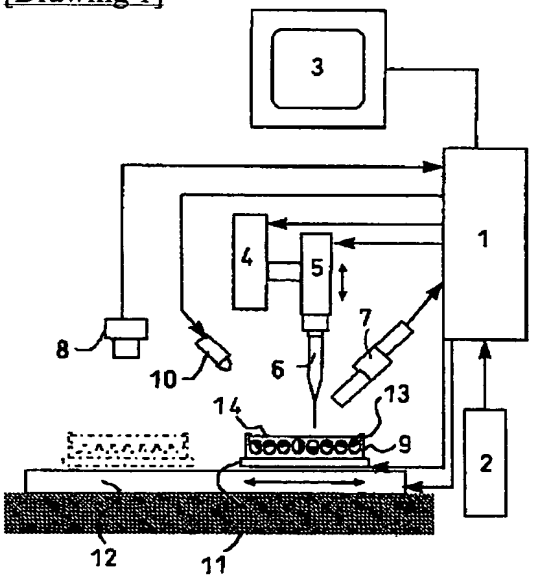
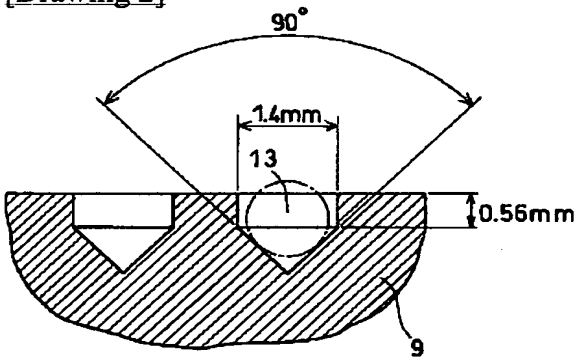
DRAWINGS

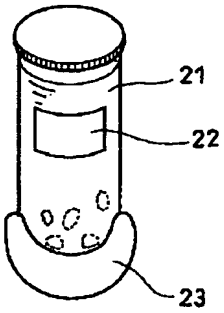
[Drawing 1]



[Drawing 2]



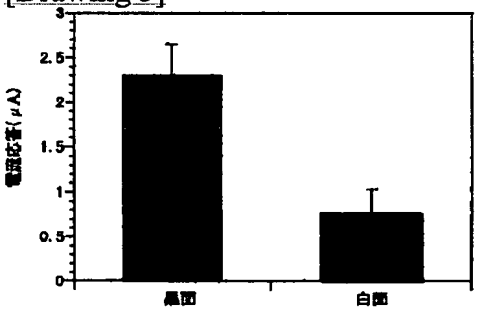
[Drawing 3]



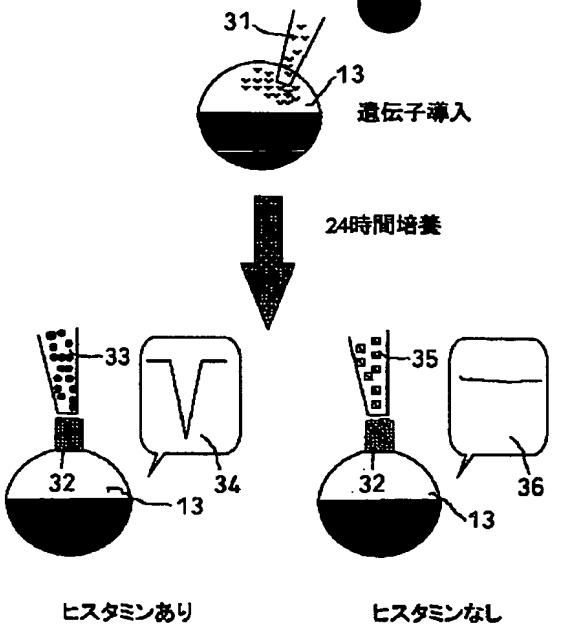
[Drawing 4]

	細胞数	反応細胞数
明	40	34
暗	28	1

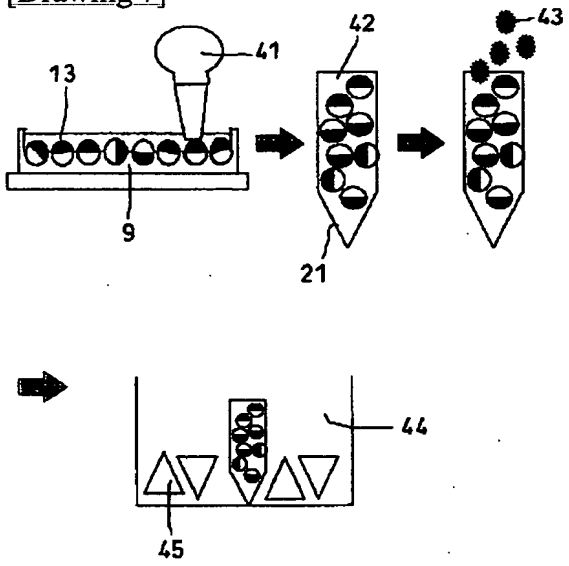
[Drawing 5]



[Drawing 6]



[Drawing 7]



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[Document to be Amended] Specification
[Item(s) to be Amended] Claim
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[Claim(s)]
[Claim 1]
A means to install the tray which has two or more openings in which one amphibian oocyte is laid,
The introductory needle which introduces a sample into the above-mentioned amphibian oocyte,
The above-mentioned tray and the mechanical component to which the relative position of the above-mentioned introductory needle is moved,
It has the control section which is made to input the depth of the above-mentioned introductory needle to the above-mentioned tray or the above-mentioned amphibian oocyte in the case of sample installation, and controls the above-mentioned migration,
Opening of the above-mentioned opening is amphibian oocyte sample installation equipment characterized by having the magnitude which permits rotating freely so that the stable direction where the above-

mentioned amphibian oocyte followed its center of gravity may be taken.
[Claim 2]
For a plane cylinder or a plane pars basilaris ossis occipitalis, a base is [the above-mentioned opening / a diameter at the maximum equator] amphibian oocyte sample installation equipment according to claim 1 to which it is characterized by being 1.4 to 2 mm in a cone form.
[Claim 3]
For a plane cylinder or a plane pars basilaris ossis occipitalis, the base of the above-mentioned opening is amphibian oocyte sample installation equipment according to claim 1 with which it is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form.
[Claim 4]
Amphibian oocyte sample installation equipment given in any 1 term of claims 1-3 characterized for the surface location of the oocyte on the above-mentioned tray by the thing of vision information, pressure variation, a temperature change, an electric change, humidity, and pH change which any one detects at least.
[Claim 5]
The above-mentioned sample is amphibian oocyte sample installation equipment given in claims 1-4 characterized by being a gene or protein.
[Claim 6]
The above-mentioned sample is amphibian oocyte sample installation equipment given in claims 1-5 characterized by including a fluorescent material.
[Claim 7]
A means to install the tray which has two or more openings in which one amphibian oocyte is laid,
The introductory needle which introduces a sample into the above-mentioned amphibian oocyte,
The above-mentioned tray and the mechanical component to which the relative position of the above-mentioned introductory needle is moved,
The control section which controls the above-mentioned migration,
The information acquisition section which acquires the vision information on the above-mentioned amphibian oocyte at the time of installation,
It has the storage section which accumulates the above-mentioned information,
Opening of the above-mentioned opening is an amphibian oocyte sample installation system characterized by having the magnitude which permits rotating freely so that the stable direction where the above-mentioned amphibian oocyte followed its center of gravity may be taken.
[Claim 8]
For a plane cylinder or a plane pars basilaris ossis occipitalis, a base is [the above-mentioned opening / a diameter at the maximum equator] the amphibian oocyte sample installation system according to claim 7 to which it is characterized by being 1.4 to 2 mm in a cone form.
[Claim 9]
For a plane cylinder or a plane pars basilaris ossis occipitalis, the base of the above-mentioned opening is the amphibian oocyte sample installation system according to claim 7 by which it is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form.
[Claim 10]
It is the tray which has two or more openings in which one amphibian oocyte is laid, and the tray which has the magnitude which permits rotating opening of the above-mentioned opening freely so that the stable direction where the above-mentioned amphibian oocyte followed its center of gravity may be taken, and the equipment which has the introductory needle which introduces a sample into the above-mentioned amphibian oocyte are used,
The process which sets the depth of the above-mentioned tray or the above-mentioned introductory needle to the above-mentioned amphibian oocyte as the 1st depth,
The process which introduces a sample into the 1st oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle at the 1st depth of the above,
The above-mentioned tray and the process to which the relative position of the above-mentioned

introductory needle is move[redacted]tomatically,
The amphibian oocyte sample installation approach characterized by having the process which introduces a sample into the 2nd oocyte of two or more above-mentioned amphibian oocytes with the above-mentioned introductory needle continuously at the 1st depth of the above.
[Claim 11]
For a plane cylinder or a plane pars basilaris ossis occipitalis, a base is [the above-mentioned opening / a diameter at the maximum equator] the amphibian oocyte sample installation approach according to claim 10 that it is characterized by being 1.4 to 2 mm, in a cone form.
[Claim 12]
For a plane cylinder or a plane pars basilaris ossis occipitalis, the base of the above-mentioned opening is the amphibian oocyte sample installation approach according to claim 10 that it is characterized by a diameter at the maximum equator being 105-150% of a diameter of two or more above-mentioned amphibian oocytes in a cone form.

[Translation done.]

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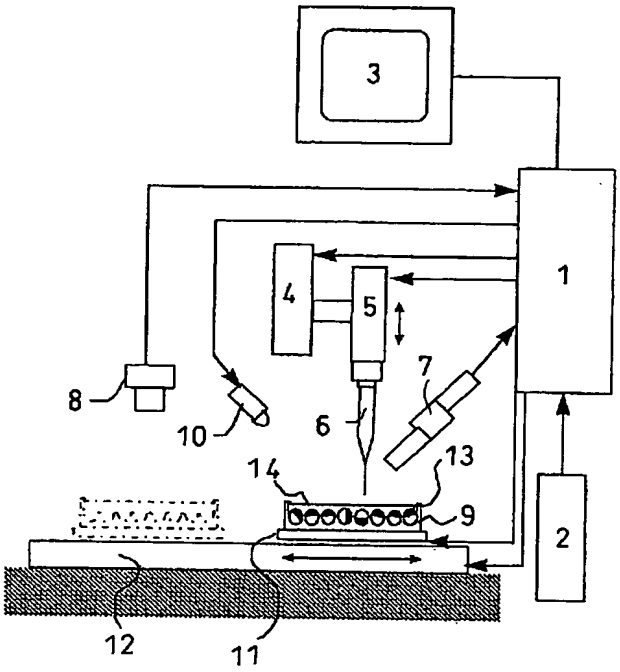
最終頁に続く

(54)【発明の名称】　両生類卵母細胞試料導入装置

(57)【要約】
【解決手段】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針と、上記トレイと上記導入針の相対位置を移動させる駆動部と、試料導入の際の上記トレイ若しくは上記両生類卵母細胞に対する上記導入針の深度を入力させ上記移動を制御する制御部とを有し、試料を上記深度で上記複数の両生類卵母細胞に導入することを特徴とする両生類卵母細胞試料導入装置。
【効果】　本発明により両生類卵母細胞に一定の深度で精度良く、試料を導入することができると共に、卵母細胞の良否、あるいは針が挿入された位置を情報として保存することができる。



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【特許請求の範囲】

【請求項１】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針と、上記トレイと上記導入針の相対位置を移動させる駆動部と、試料導入の際の上記トレイ若しくは上記両生類卵母細胞に対する上記導入針の深度を入力させ上記移動を制御する制御部とを有し、試料を上記深度で上記複数の両生類卵母細胞に導入することを特徴とする両生類卵母細胞試料導入装置。

【請求項２】　上記駆動部は、上記トレイと上記導入針の相対位置を３次元方向に駆動させることを特徴とする、請求項１に記載の両生類卵母細胞試料導入装置。

【請求項３】　さらに試料導入時の上記両生類卵母細胞の視覚情報取得部を有することを特徴とする請求項1又は２に記載の両生類卵母細胞試料導入装置。

【請求項４】　上記視覚情報取得部はカメラであることを特徴とする請求項３に記載の両生類卵母細胞試料導入装置。

【請求項５】　上記視覚情報取得部によって得られた上記両生類卵母細胞各々の視覚情報を各細胞の上記トレイ上の位置と関連づける手段をさらに有する、請求項３又は４に記載の両生類卵母細胞試料導入装置。

【請求項６】　上記視覚情報を記憶する記憶部を更に有することを特徴とする、請求項３から５のいずれか１項に記載の両生類卵母細胞試料導入装置。

【請求項７】　上記トレイは、上記複数の両生類卵母細胞を保持するための複数の穴を有することを特徴とする、請求項１から６のいずれか１項に記載の両生類卵母細胞試料導入装置。

【請求項８】　上記穴は底面が平面の円筒又は底部が円錐形で最大直径が１．４－２ｍｍであることを特徴とする、請求項７に記載の両生類卵母細胞試料導入装置。

【請求項９】　上記穴の底面が平面の円筒又は底部が円錐形で最大直径は上記複数の両生類卵母細胞の直径の１０５－１５０％であることを特徴とする、請求項７に記載の両生類卵母細胞試料導入装置。

【請求項１０】　上記トレイ上の卵母細胞の表面位置を視覚情報、圧力変化、温度変化、電気変化、湿度変化、ｐＨ変化の少なくともいずれか１つによって検出することを特徴とする、請求項１から９のいずれか１項に記載の両生類卵母細胞試料導入装置。

【請求項１１】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針と、上記トレイと上記導入針の相対位置を移動させる駆動部と、上記移動を制御する制御部と、導入時の上記両生類卵母細胞の視覚情報を得る情報取得部と、上記情報を蓄積する記憶部とを有し、上記試料を上記複数の両生類卵母細胞に導入することを特徴とする両生類卵母細胞試料導入システム。

【請求項１２】　上記トレイは、上記複数の両生類卵母細胞を保持するための複数の穴を有することを特徴とする、請求項１１に記載の両生類卵母細胞試料導入システム。

【請求項１３】　上記穴の底面が平面の円筒又は底部が円錐形で最大直径は上記複数の両生類卵母細胞の直径の１０５－１５０％であることを特徴とする、請求項１２に記載の両生類卵母細胞試料導入システム。

【請求項１４】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記トレイ若しくは上記両生類卵母細胞に対する上記導入針の深度を第1の深さに設定する工程と、上記複数の両生類卵母細胞のうちの第1の卵母細胞に上記導入針で試料を上記第1の深さに導入する工程と、上記トレイと上記導入針の相対位置を自動的に移動させる工程と、続けて上記複数の両生類卵母細胞のうちの第2の卵母細胞に上記導入針で上記第1の深さに試料を導入する工程とを有することを特徴とする、両生類卵母細胞試料導入方法。

【請求項１５】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記複数の両生類卵母細胞のうちの第1の卵母細胞に上記導入針で試料を導入する工程と、上記トレイと上記導入針の相対位置を移動させる工程と、続けて上記複数の両生類卵母細胞のうちの第2の卵母細胞に上記導入針で試料を導入する工程と、上記試料導入時の卵母細胞の状態を視覚情報として入手する工程と、上記視覚情報を蓄積する工程とを有することを特徴とする、両生類卵母細胞試料導入方法。

【請求項１６】　上記試料は遺伝子又は蛋白質であることを特徴とする、請求項１４又は１５に記載の両生類卵母細胞試料導入方法。

【請求項１７】　上記試料は蛍光物質を含むことを特徴とする、請求項１４から１６のいずれか１項に記載の両生類卵母細胞試料導入方法。

【請求項１８】　上記第1の卵母細胞に導入する試料と、上記第2の卵母細胞に導入する試料の導入量を実質的に同一にするよう制御することを特徴とする、請求項１４から１７のいずれか１項に記載の両生類卵母細胞試料導入方法。

【請求項１９】　試料の導入深度が実質的に等しい条件でそれぞれ上記試料が導入されたことを特徴とする、複数の両生類卵母細胞。

【請求項２０】　さらに導入位置が実質的に同じ条件で上記試料が導入されたことを特徴とする、請求項１９に記載の両生類卵母細胞。

【請求項２１】　さらに上記試料の導入量が実質的に一定であることを特徴とする、請求項１９又は２０に記載の複数の両生類卵母細胞。

【請求項２２】　試料の導入深度が実質的に等しい条件でそれぞれ上記試料が導入されたことを特徴とする複数

の両生類卵母細胞を内部に有し、上記導入の日時に関する情報と、上記卵母細胞の使用期限に関する情報が付されていることを特徴とする容器。

【請求項２３】　さらに上記試料の導入位置又は上記試料の導入量の少なくともいずれか１つについても実質的に等しい条件で導入されている上記複数の両生類卵母細胞を内部に有することを特徴とする、請求項２２に記載の容器。

【請求項２４】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記トレイに対する上記導入針の相対的位置を移動させながら、上記複数の両生類卵母細胞の各々に上記導入針で試料を導入し、上記導入時の上記複数の両生類卵母細胞各々の視覚情報を入手し、上記視覚情報に基づいて上記複数の両生類卵母細胞のうち導入が卵母細胞の黒面に導入された複数の卵母細胞を収集することを特徴とする、試料が導入された両生類卵母細胞の製造方法。

【請求項２５】　複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記トレイに対する上記導入針の相対的位置を移動させながら、上記複数の両生類卵母細胞の各々に上記導入針で試料を導入し、上記導入時の上記複数の両生類卵母細胞各々の視覚情報を入手し、上記視覚情報に基づいて上記複数の両生類卵母細胞のうち導入が卵母細胞の白面に導入された複数の卵母細胞を収集することを特徴とする、試料が導入された両生類卵母細胞の製造方法。

【請求項２６】　上記導入前に上記両生類卵母細胞若しくは上記トレイに対する上記導入針の深度を第1の深さに設定し、上記複数の両生類卵母細胞には上記第1の深さで試料の導入を行うことを特徴とする、請求項２４又は２５に記載の両生類卵母細胞群製造方法。

【請求項２７】　試料の導入深度が実質的に等しい条件で試料がそれぞれ導入された複数の両生類卵母細胞をセットにすることを特徴とする、複数の両生類卵母細胞を販売又は譲渡する方法。

【請求項２８】　上記セットに上記複数の両生類卵母細胞への試料導入に関する情報を添付することを特徴とする、請求項２７に記載の複数の両生類卵母細胞を販売又は譲渡する方法。

【請求項２９】　試料の導入深度が実質的に等しい条件でそれぞれ導入された複数の両生類卵母細胞を容器に入れ、上記容器に上記複数の両生類卵母細胞への試料導入に関する情報を記したラベルを添付して複数の両生類卵母細胞を販売又は譲渡する方法。

【請求項３０】　上記容器の温度は１８℃以上２２℃以下に調節されていることを特徴とする、請求項２９に記載の複数の両生類卵母細胞を販売又は譲渡する方法。

【請求項３１】　上記複数の両生類卵母細胞への試料導入に関する情報は、導入の日時、卵母細胞の品質保証期間、試料を導入した位置、試料を導入した深度、発現の確率の少なくともいずれか１つに関するものであることを特徴とする、請求項２７から３０のいずれか１項に記載の複数の両生類卵母細胞を販売又は譲渡する方法。

【請求項３２】　遺伝子又は蛋白質をほぼ一定な位置かつ深度に導入した複数の両生類卵母細胞をスクリーニング用のセンサーとして用いる方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明はカエル等の両生類の卵母細胞に遺伝子、色素、蛋白質、ペプチド、薬物等の試料をピペット様の針を用いて導入する自動化装置に関する。また遺伝子、色素、蛋白質、ペプチド、薬物等の試料を両生類卵母細胞の特定位置に導入する方法、試料の導入に関して質の保証された両生類卵母細胞、並びに特定位置及び深度に試料を導入した両生類卵母細胞を販売又は譲渡する方法に関する。

【０００２】

【従来の技術】遺伝子、色素、蛋白質、ペプチド、薬物等の試料を導入したアフリカツメガエル等のカエルの卵母細胞は、サイズが比較的大きく、また低コストで多量に得られるため、生細胞への色素、蛋白質、ペプチド、薬物などの作用の確認、遺伝子機能の解析、遺伝子産物としての蛋白質の生産などの目的で広く使用されているが、従来は個々の研究者がカエルを飼育し、卵母細胞を採取することから行っていた。

【０００３】従来、遺伝子、色素、蛋白質、ペプチド、薬物等の試料をカエル等の両生類の卵母細胞に導入する際には、これらの試料を充填したピペットをマニピュレーターを用い、顕微鏡下にて、技術者が手動で卵母細胞に刺入していた。ピペットはインジェクターに装着し、油圧または空気圧などで細胞内に一定量の試料を吐出する。また、電圧を印可して細胞内に一定量の試料を吐出する方法として、特開平5-192171号や特開平6-343478号がある。いずれの方法も顕微鏡下で細胞を観察しながら、手動にて細胞に針を近づけ、試料の導入を行うという技術である。

【０００４】

【発明が解決しようとする課題】上記の手動による遺伝子等の導入は、技術者個人の技量、熟練度等によって、試料が導入できた卵母細胞の割合が変動するという問題があった。これは技術者毎に一定時間当たりの試料導入可能な卵母細胞数が異なるため、試料の継時的な変成度が技術者間でばらつくことが原因の一つである。本発明は技術者の技量、熟練度に関わりなく、時間当たりの処理数を一定にすることを目的の一つとしている。

【０００５】また上記の従来技術は卵母細胞への試料導入深度を統一化することへの配慮がされておらず、熟練した技術者でも深度を制御することは困難であった。そ

の結果、核など特定の細胞内小器官への試料導入は偶然に頼ることになっていた。本発明は、試料導入深度を統一化することにより、核などへの深さ方向での制御が可能な細胞内小器官への試料導入を容易にすることを目的の一つとしている。

【０００６】さらに上記の従来技術では、試料を導入した際の細胞の情報を保存することへの配慮がされておらず、試料導入情報とその後の細胞反応との相関を得ることが困難であった。従って本発明の目的はこれらの相関を得ることにある。

【０００７】また、従来は卵母細胞を個々に調達していたため、大量生産や需要に応じたタイミングの良い生産は不可能であった。従って本発明の他の目的は、特定の試料を導入した卵母細胞、または特定の試料を一定の位置に導入したことを保証した卵母細胞を、生産・販売及び輸送することにある。

【０００８】

【課題を解決するための手段】本発明は上記課題を解決するために、ピペット様の針を用いて遺伝子、色素、蛋白質、ペプチド、薬物等の試料をカエル等の両生類の卵母細胞の任意の位置、任意の深度に自動制御にて導入する装置を提供するものである。

【０００９】すなわち本発明は、複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針と、上記トレイと上記導入針の相対位置を移動させる駆動部と、試料導入の際の上記トレイ若しくは上記両生類卵母細胞に対する上記導入針の深度を入力させ上記移動を制御する制御部とを有し、試料を上記深度で上記複数の両生類卵母細胞に導入することを特徴とする両生類卵母細胞試料導入装置を提供する。

【００１０】また、本発明は、複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針と、上記トレイと上記導入針の相対位置を3次元方向に移動させる駆動部と、上記移動を制御する制御部と、導入時の上記両生類卵母細胞の視覚情報を得る情報取得部と、上記情報を蓄積する記憶部とを有し、上記試料を上記複数の両生類卵母細胞に導入することを特徴とする両生類卵母細胞試料導入システムを提供する。これにより、複数の両生類卵母細胞に対し、ほぼ一定の深度に試料を速く導入することが可能になった。

【００１１】さらに、上記トレイは、底面が平面の円筒又は底部が円錐形で最大直径は上記複数の両生類卵母細胞の直径の１０５－１５０％であることを特徴とする穴を有する。これにより、他の特別な手段を用いることなく、トレイ上の複数の卵母細胞のほぼ８割について同一面への導入が可能になった。

【００１２】本発明者等は、卵母細胞にmRNAを導入した場合、卵母細胞の黒面にmRNAを導入した場合と白面にmRNAを導入した場合では、蛋白質の機能発現効率が異なることを見出した。つまり、卵母細胞間での蛋白質の機能発現効率のばらつきを抑えるためには、同じ面にmRNAを導入した卵母細胞を集めることが重要となる。本発明では、試料を導入した際の細胞の情報を保存し、その後の細胞反応との相関を容易に導き出すことを可能にするものである。

【００１３】さらに、本発明は、複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記トレイ若しくは上記両生類卵母細胞に対する上記導入針の深度を第1の深さに設定する工程と、上記複数の両生類卵母細胞のうちの第1の卵母細胞に上記導入針で試料を上記第1の深さに導入する工程と、上記トレイと上記導入針の相対位置を自動的に移動させる工程と、続けて上記複数の両生類卵母細胞のうちの第2の卵母細胞に上記導入針で上記第1の深さに試料を導入する工程とを有することを特徴とする、両生類卵母細胞試料自動導入方法を提供する。

【００１４】さらにまた本発明は、複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記複数の両生類卵母細胞のうちの第1の卵母細胞に上記導入針で試料を導入する工程と、上記トレイと上記導入針の相対位置を移動させる工程と、続けて上記複数の両生類卵母細胞のうちの第2の卵母細胞に上記導入針で試料を導入する工程と、上記試料導入時の卵母細胞の状態を視覚情報として入手する工程と、上記視覚情報を蓄積する工程とを有することを特徴とする、両生類卵母細胞試料自動導入方法を提供する。

【００１５】上記両生類卵母細胞試料導入装置または導入方法の発明により、本発明はさらに、試料の導入深度が実質的に等しい条件でそれぞれ上記試料が導入されたことを特徴とする、複数の両生類卵母細胞を提供する。また、本発明は、さらに導入位置が実質的に同じ条件で上記試料が導入されたことを特徴とする、上記の両生類卵母細胞を提供する。

【００１６】さらに、本発明は以下のものを提供する。複数の両生類卵母細胞を保持するトレイと、上記複数の両生類卵母細胞に試料を導入する導入針とを有する装置を用い、上記トレイに対する上記導入針の相対的位置を移動させながら、上記複数の両生類卵母細胞の各々に上記導入針で試料を導入し、上記導入時の上記複数の両生類卵母細胞各々の視覚情報を入手し、上記視覚情報に基づいて上記複数の両生類卵母細胞のうち導入が卵母細胞の黒面に導入された複数の卵母細胞、又は卵母細胞の白面に導入された複数の卵母細胞を収集することを特徴とする、試料が導入された両生類卵母細胞群の製造方法。

【００１７】試料の導入深度が実質的に等しい条件で試料がそれぞれ導入された複数の両生類卵母細胞をセットにし、上記複数の両生類卵母細胞への試料導入に関する情報を添付することを特徴とする、複数の両生類卵母細

胞を販売又は譲渡する方法。

【００１８】又は、試料の導入深度が実質的に等しい条件でそれぞれ導入された複数の両生類卵母細胞を容器に入れ、上記容器に上記複数の両生類卵母細胞への試料導入に関する情報を記したラベルを添付して複数の両生類卵母細胞を販売又は譲渡する方法。

【００１９】ここで、上記複数の両生類卵母細胞への試料導入に関する情報は、導入の日時、卵母細胞の品質保証期間、試料を導入した位置、試料を導入した深度、発現の確率の少なくともいずれか１つに関するものである。よって、本発明により、導入に関する条件を実質的に同じくする複数の卵母細胞群を、それらの情報と共に得ることが可能になった。

【００２０】

【発明の実施の形態】以下に、図面を参照しながら本発明を更に説明する。図１に、この装置の原理を示す。卵母細胞を整列配置させるトレイは、例えば水平方向に12個、直行方向に8個の計96個の深さ及び形状が均一な穴の開いたトレイが使用できるが、トレイの穴数は必ずしも96個には限定しない。両生類卵母細胞は一般に白面の重量が黒面に比して大きい。よってトレイの穴径を用いる卵母細胞より多少大きくすることで、トレイに整列した卵母細胞は回転等によって向きを変えることなく、平均して約８割の細胞は黒面を上にして保持され、他に特別な手段を用いることなく同一面への導入確率を上げることができる。卵母細胞を整列させるための穴の形状は、底面が円形の平面であって底面から開口部までの底面に平行な断面形状が一定である円筒形、又は底部が円錐形の形が好ましい。上記穴の開口部の直径は、用いる両生類卵母細胞の直径以上である必要がある。また上気した理由から生理食塩水等を満たした上記穴中で卵母細胞が回転できる程度の余地があることが望ましく、具体的には用いる卵母細胞直径の１０５－１５０％を穴の最大直径とすることが望ましいことを実験的に確かめている。例えばアフリカツメガエルの卵母細胞の直径が約1.3mm程度であることから、穴の直径を1.4～2mm程度とすることにより、細胞を傷つけることなく、特定の方向に固定することが可能になる。本発明において好適な穴の形状は、例えば図２に示すような、底の角度90°、直径1.4mm、円柱部分の深さ0.56mmのすり鉢型のものである。また、上記トレイを用いる他にスポイト等を使用して、同一面への導入確率をさらに上げたり、また特定の面、例えば白面が上になるように操作することもできる。

【００２１】導入する試料としては、遺伝子、色素、蛋白質、ペプチド、薬物等が挙げられ、特に限定されるものではない。また、下記の実施例ではヒトヒスタミン受容体cRNAを導入した卵母細胞について記載するが、導入に使用する遺伝子はcRNAに限定するものではなく、DNA及びRNA、合成したオリゴヌクレオチドであっても良い。試料を導入するための針は、ピペット様の針であることが好ましいが、特に限定するものではない。また、卵母細胞の向き等の細胞情報を収得するためにデジタルカメラ8を、導入針6が卵母細胞表面が接触したことを検知するための手段として、CCDカメラ7を通した視覚情報を例としているが、これらの情報を得るために必要な手段はデジタルカメラ8やCCDカメラ7に限定されるものではない。例えば、圧力、温度、電気、湿度、ｐＨ等の変化を感知するセンサーを導入装置にとりつけることで、これらの情報を基に細胞表面を検知することができる。

【００２２】トレイ9の穴に試料導入前の卵母細胞を並べ、トレイ9に両生類用生理食塩水14を満たした後、直行移動台11と水平移動台12の上に設置する。この直行移動台11と水平移動台12の移動を制御装置１によりＸ軸方向及びＹ軸方向に制御することによって、導入針６から試料を導入する卵母細胞13の位置を決定することが好ましいが、図1の構成と逆に、トレイ9を固定し、導入針6を移動可能な構成とすることもできる。

【００２３】トレイ9が破線の位置にあるとき、デジタルカメラ8で卵母細胞を撮影し、制御装置1にその撮影データを送ることにより、卵母細胞の良否及び向きなどの情報を蓄積することができる。

【００２４】水平移動台12と直行移動台11を制御装置1の指示で動作し、トレイ9に並べられた卵母細胞のうち所定の位置にある最初の卵母細胞13の中心を遺伝子導入針6の下方位置に移動する。ここで導入針のＺ軸方向の移動のために導入針移動台4が制御装置1の指示で動作し、導入装置5に装着された導入針6の先端を卵母細胞の表面よりわずかに離れた位置、例えば数100mm手前まで下降させる。ここで、CCDカメラ7で撮影された画像をモニター3で観察しながら、制御補助装置2で指令を出し、導入針移動台4を低速で下降させる。視覚情報、圧力変化、温度変化、電気変化、湿度変化、pH変化等で卵母細胞13の表面に導入針6の先端が接触したことを検知し、この位置で導入針移動台4を止める。この位置が以降の遺伝子導入操作の基準点となる。この位置を制御装置1に記憶させ、以下の操作を実施する。すなわち、上記基準点から試料を導入する位置までの、トレイの置かれている面に対して垂直方向の導入針の移動距離、深度を設定し、上記設定された深度で導入針6を刺入して試料を一定量吐出させる。試料導入のためには、例えば上記の卵母細胞13表面に導入針6が接触した位置から0.2mm下方に導入針を下げるといったＺ軸制御を行うことができる。細胞への導入針6の最適な導入深度は、導入する試料の種類や導入の目的により異なり、適宜設定することができる。試料は導入深度が浅すぎると細胞中に広がらず、又深すぎると核を傷つけたり細胞を傷める確率が高くなる。よって、試料の導入にはほぼ一定の深度に導入することが発現効率の点からも望ましい。例えば、細胞質内にmRNAを導入し、蛋白質を発現させたい場合には、

細胞表面から0.02～0.1mmの深度に針を刺入することが望ましい。一方、核内にDNAを導入し、蛋白質を発現させたい場合には、細胞表面から0.05～0.2mmの深度に針を刺入することが望ましい。但し、導入時に卵母細胞の形状が針の接触によりわずかにゆがむため、実際には設定した深度よりも浅い位置に試料が導入される。試料導入のための時間は、細胞への注入量等に応じて、針が細胞内に注入されている時間を設定することにより制御する。尚、導入効率を更に向上させるために、導入針6は複数個にすることもできる。この場合、装置の駆動部は、導入針とトレイの相対的位置を１次元又は２次元方向に移動させることで十分とすることもできる。

【００２５】この後、最初の卵母細胞の3次元的な位置情報を基に、自動制御によりトレイ9内の二つ目以降の任意の数の卵母細胞に、指示された時間と速度及び導入深度で試料を自動的に導入する。また、卵母細胞の大きさにばらつきがある場合があるため、導入の都度表面位置検出を行う機能も備えさせることもできる。また、卵母細胞の細胞情報をコンピュータに記憶させ、必要時にそれを引き出すこともできる。

【００２６】更に、試料導入時の上記導入針6及び卵母細胞13の動きや、導入時の卵母細胞の視覚情報等をコンピュータに記憶させ、導入操作終了後に試料導入位置、深度、細胞の特徴等を読み出すように設定することもできる。この場合、各卵母細胞に関する視覚情報は番号を付す等することによって上記トレイ上の位置と関連付けて行うことが望ましい。

【００２７】また、両生類の卵母細胞には白面及び黒面があることが知られており、それぞれ機能が異なっていることが知られていたが、本発明者等は、卵母細胞にmRNAを導入した場合、卵母細胞の黒面にmRNAを導入した場合と白面にmRNAを導入した場合では、蛋白質の機能発現効率が異なることを見出した。すなわち、ヒスタミン受容体mRNAを導入する場合には、黒面に導入した方が、白面に導入した場合よりも発現効率が高く、リガンド応答性の良い卵母細胞を得ることができる。一方、発色団を含む蛋白質、蛍光蛋白質、またはこれらの遺伝子、色素を導入する場合には、白面に導入した方が色や光の情報を感度高く得ることができる。上記したように上記トレイを用いることでトレイ上の細胞については約８割について黒面への導入が可能である。あるいは、細胞の特定面、例えば黒面のみ、または白面のみに試料を導入した細胞を得たい場合には、上記したように、試料導入前に予め特定の面のみが上になるように個々の細胞をスポイト等を用いて操作する。若しくは、試料を導入する際に視覚情報検出手段や白黒判別センサーにより細胞表面の試料導入位置情報を収得し、収得された情報から、目的の面が上になった細胞のみに試料が導入された細胞を収集することもできる。これにより導入位置の条件を実質的に同じくする卵母細胞群が得られる。本発明において、「特定の位置」とは、例えば卵母細胞の黒面側、白面側、又は赤道付近等の位置をいう。

【００２８】上記の構成の装置を使用することにより、両生類卵母細胞の特定位置及び深度に試料を導入することができ、導入された試料の機能発現効率（導入効率）がほぼ同品質の卵母細胞を、迅速に、かつ大量に生産することができる。従って、本発明は、本発明の装置を使用して両生類卵母細胞の特定の位置及び深度に試料を導入する方法も提供する。

【００２９】本発明の装置を使用することにより、試料導入効率は次のように向上することがわかった。すなわち、手動操作による試料導入の経験がない初心者の場合、25個の細胞に試料を導入するために約30分程度かかり、導入効率は、遺伝子を試料とした場合の発現率で約30％であるが、本発明の装置の使用により、25個の細胞に試料を導入するための時間はわずか３分間となり、導入効率は80％程度にまで達する。手動操作による試料導入の熟練者の場合には、導入のためにかかる時間にそれ程の短縮は見られないが、導入効率は、例えば手動で80％である場合には装置の使用により更に上昇して90％にまで達することができる。

【００３０】従って、本発明の装置及び方法を使用することにより、操作者の技量に依存することなく、約80～90％の導入効率が達成され、本発明により、試料導入条件の制御された複数の卵母細胞を販売又は譲渡することが初めて可能になったのである。従って別の観点において、本発明は特定の位置及び深度への試料の導入が保証された両生類卵母細胞を提供する。

【００３１】また、特定の位置及び深度に試料を導入した卵母細胞のみを収集し、販売又は譲渡することができる。販売又は譲渡の際には、複数個の卵母細胞をパッケージングし、試料の種類、導入日時、品質の保証期間、試料を導入した位置及び深度に関する設定条件等の情報、保証される導入効率等を記載したラベル22を付すことができる（図３）。

【００３２】さらに、本発明は、特定の深度さらに位置、又導入した遺伝子がコードする蛋白質の発現等に関する情報も付して、卵母細胞を販売又は譲渡することができる。試料導入効率、又、発現効率を保証した卵母細胞を販売・譲渡する際は、例えば色素又は発色団を含む蛋白質や蛍光蛋白質、またはこれらの蛋白質をコードする遺伝子を試料と共導入し、有色又は蛍光を発する卵母細胞数を数え、その割合を試料導入効率の指標として、試料導入効率を保証することができる。共導入は混合形態で行っても良いが、共導入する試料及び検出のための蛋白質等が共に遺伝子の形態である場合は、融合蛋白質をコードする遺伝子とすることもできる。

【００３３】試料の導入効率又は発現効率を、共導入した蛍光蛋白質からの蛍光を指標に算定する例について、図４を用いて説明する。本実施例では、オワンクラゲ由

来緑色蛍光蛋白質（GFP）の発現を指標にヒスタミン受容体遺伝子の導入割合を検定した例について述べるが、本発明は導入する試料を遺伝子に限定するものではない。また、試料の導入効率の指標として用いる物質も、GFPに限定するものではない。

【００３４】上記機構を備えた装置、または上記原理を用いて、卵母細胞の白面にヒスタミン受容体遺伝子と緑色蛍光蛋白質遺伝子の混合物を導入する。遺伝子導入操作後、24時間経過した卵母細胞に波長488nmの光を照射すると、緑色蛍光蛋白質が発現したものは507nmの蛍光を発する。507nmの蛍光を発した細胞を明、蛍光を発さなかった細胞を暗として分類する。さらに、これらの細胞をヒスタミン刺激し、応答の有無で分類したものが図４である。図４に示したように、明の細胞では、85%の細胞（40個中34個）がヒスタミンに応答した。すなわち85%以上の細胞にヒスタミン受容体遺伝子が導入できている。逆に暗の細胞では、90%以上の細胞がヒスタミンに応答しなかった（28個中27個）。すなわち90%以上の細胞にヒスタミン受容体遺伝子が導入できていない。つまり、緑色蛍光蛋白質が発現している卵母細胞は、ヒスタミン受容体遺伝子も導入されている割合が高いことが明らかになった。

【００３５】このことから明らかなように、目的の試料を蛍光蛋白質等と共導入すれば、その蛍光等の有無を指標として目的の試料の導入効率を算定することができ、試料導入効率を保証して卵母細胞を販売または譲渡することが可能である。

【００３６】次に、ヒトヒスタミン受容体cRNAを導入した卵母細胞を用いた例を用い、特定の用途のために本発明の方法によって得られた卵母細胞を生産し、販売又は譲渡する手段について記載するが、導入に使用する遺伝子はcRNAに限定するものではなく、またDNA及びRNA、合成したオリゴヌクレオチドであっても良い。

【００３７】上記の本発明に係る装置及び方法を使用して、卵母細胞にヒスタミン受容体cRNAを導入する。この際、白黒判別センサまたはCCDカメラ/デジタルカメラなど視覚情報を得る手段を用いれば、黒面にcRNAを導入した卵母細胞と、白面にcRNAを導入した卵母細胞を分別することができる。

【００３８】遺伝子導入操作後、24時間以内で卵母細胞にヒスタミン受容体が発現する。ヒスタミン受容体遺伝子導入操作後、２４時間以上経て、ヒスタミン受容体が発現したと考えられる卵母細胞の膜電位を二電極膜電位固定法により、−60mVに固定する。このような条件下において、卵母細胞にヒスタミンを含んだ試料を添加すると、試料中のヒスタミンとヒスタミン受容体が相互作用し、卵母細胞内の情報伝達系が活性化し、イオン流が発生するため、卵母細胞はヒスタミンに対して電気的応答を示す。24時間を経た卵母細胞を1μMヒスタミンで刺激し、その電流応答を測定する。黒面または白面に遺伝子を導入した卵母細胞の電流応答の大きさを比較したものが図５である。図５に示したように、遺伝子導入部位により、ヒスタミン刺激への電流応答の違いが見られた。つまり、黒面に遺伝子を導入した場合の方が、白面に遺伝子を導入した場合よりも、よりリガンド応答性の良い卵母細胞が得られることが示された。

【００３９】従って、例えば黒面のみにヒスタミン受容体cRNAを導入された卵母細胞集団は、ヒスタミンに対する感度の良いセンサとして使用することができる。さらに、本発明により、特定の位置及び深度に試料を導入した卵母細胞をセンサに用いることを目的として販売又は譲渡することができる。当業者には容易に認識されるように、本発明によって得られる卵母細胞をセンサとして使用するために導入し得る試料は、ヒスタミン以外の任意のリガンドに対する受容体の遺伝子、特定の抗原に対する反応性を有する抗体、特定の糖鎖を有する糖蛋白質等が挙げられ、特に限定されるものではない。

【００４０】上記機構を備えた装置、または上記原理を用いて、卵母細胞13にヒスタミン受容体遺伝子31を導入する。図６には便宜的に白面に導入する場合について記載した。ヒスタミン受容体遺伝子導入操作後、24時間以内で卵母細胞13にヒスタミン受容体32が発現する。上記と同様に、ヒスタミン受容体遺伝子導入操作後、２４時間以上経て、ヒスタミン受容体32が発現したと考えられる卵母細胞の膜電位を二電極膜電位固定法により、−60mVに固定する。このような条件下において、卵母細胞13にヒスタミンを含んだ試料33を添加すると、試料中のヒスタミンとヒスタミン受容体が相互作用し、卵母細胞内の情報伝達系が活性化し、イオン流が発生するため、卵母細胞13はヒスタミンに対して電気的応答34を示す。ヒスタミンを含まない試料35を添加した場合は、受容体と相互作用する物質が存在しないため、卵母細胞13はヒスタミンに対して応答しない36。

【００４１】すなわち、ヒスタミン受容体遺伝子を導入した卵母細胞に試料を添加し、その細胞応答の有無を指標にして、試料中のヒスタミンの有無をセンシングすることができる。従って本発明である試料導入装置を用いることで試料の導入条件を同じくする卵母細胞を大量に生産できるようになったことで両生類卵母細胞をある受容体、抗体に反応するリガンド、又は抗原等のスクリーニング用に用いることが可能になった。すなわち、実質的に同一な条件のもとで遺伝子等の導入を行い、蛋白質等を発現させた複数の卵母細胞各々に異なるリガンド等を反応させることでその結果を比較し、スクリーニングを行うことができる。

【００４２】また、別の用途として、蛋白質等を発現している卵母細胞をつぶす等することによって発現している蛋白質等を抽出することができるが、本発明の導入装置を用いて導入条件を制御し、例えば黒面に試料を導入した細胞を用いることで効率よく所望の蛋白質等を抽出

することが可能である。

【００４３】次に、本発明に係る試料を導入した卵母細胞を輸送する方法について、図６に従って記載する。上記の卵母細胞を販売・輸送する際には、両生類の卵母細胞用に通常使用される緩衝液に、例えば硫酸ゲンタマイシン、ペニシリン、ストレプトマイシン等の抗生物質を加えた溶液を満たした容器21に卵母細胞13を入れ、発泡スチロール等の梱包材を使用し、衝撃を避けながら、図３に示すように、保冷剤23の使用等により温度を４～25℃、好ましくは18～22℃に保ち、輸送することが好ましい。

【００４４】上記溶液の組成は特に限定されるものではないが、例えば以下の組成のものを好適に使用することができる。この溶液のｐＨは７．５である。

NaCl	９６ｍＭ
KCl	２ｍＭ
$CaCl_2$	１．８ｍＭ
$MgCl_2$	１ｍＭ
HEPES	５ｍＭ
硫酸ゲンタマイシン	５０μｇ／ｍｌ
ピルビン酸ナトリウム	２．５ｍＭ
ペニシリン	１０Ｕ／ｍｌ
ストレプトマイシン	１０μｇ／ｍｌ

【００４５】販売・輸送のために好適な容器としては、容器21内で細胞が比較的自由に移動でき、開閉できる蓋のある密閉可能なものであれば特に限定されるものではなく、その容積の９割５分程度まで上記の溶液を満たすことが好ましい。例えば50mlのコニカルチューブの場合には、約100～200個の卵母細胞、好ましくは約130～180個の細胞を入れることが好ましい。この割合は卵母細胞１個に対して約0.3～0.5ml程度に相当するが、卵母細胞の種類、容器の種類等によって変動し得る。

【００４６】図７に示すように、試料を導入した卵母細胞13はトレイ9から、スポイト41等を用いて回収する。この際、先に記録した情報を基に、黒面又は白面に試料が導入された卵母細胞のみを回収することもできる。回収した卵母細胞を容器21に移す。両生類用緩衝液42を当該容器21に満たし、数回、液を交換する。交換後、再度、両生類用緩衝液42を容器21に満たし、さらに適量の抗生物質43を加える。チューブの蓋を閉め、保冷剤入りの外容器44に入れる。外容器44に梱包材45等を入れ、容器21を固定し、一般的な運搬手段で、購入先まで運搬する。

【００４７】この方法により、試料を導入した卵母細胞の機能を損なうことなく購入先まで運搬することができる。また、販売・譲渡の際には導入日時・試料を導入した場所、深度等の導入条件、再現率、品質保証期間等の情報を一緒に提供する。その方法としては例えばそれらの情報を記載した紙面を添付したり、ラベル22を複数の卵母細胞を有する容器21に添付すること等がある（図３）。

【００４８】遺伝子を導入した場合には発現までに約２４時間程度かかり、又卵母細胞の寿命としては導入後７日間程度、より好ましくは５日間程度が目安となるため、導入した日時や、それに対応して有効な導入後の期間を明示するために「○月○日までの使用が望ましい」等の記載をすることが好ましい。導入面については、上記のトレイを用いることで約８割の卵母細胞については黒面への導入が可能であり、また上記装置に蓄積される卵母細胞の情報を基に発現効率については共発現を利用してその程度を高めることも可能である。

【００４９】

【発明の効果】本発明により、カエル等の両生類の卵母細胞に一定の深度で精度良く、試料を導入することができ、導入効率等が同品質の卵母細胞を迅速かつ大量に得ることができる。卵母細胞の良否、あるいは針が挿入された位置は情報として保存できる。また、本発明の方法によって得られた、特定位置及び深度に試料を導入した卵母細胞のみを回収し、あるいは試料導入効率を保証して販売又は譲渡することが可能である。更に、導入した試料の種類に応じ、用途を特定して販売又は譲渡することもできる。

【図面の簡単な説明】

【図１】本発明の装置構成図を示す。

【図２】本発明に使用するトレイの形状の一例を示す。

【図３】本発明に使用する容器の一例を示す。

【図４】卵母細胞に導入したGFPからの蛍光の有無と、共導入により発現したヒスタミン受容体のリガンド応答性の相関性を示す。

【図５】遺伝子導入位置（黒面又は白面）と電流応答によるリガンド応答性の関係を示す。

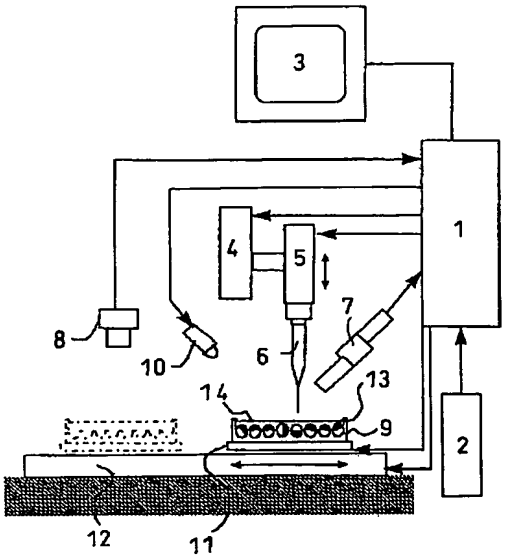
【図６】ヒスタミンセンサーとしての卵母細胞の使用を示す。

【図７】試料導入後の卵母細胞の輸送方法を示す。

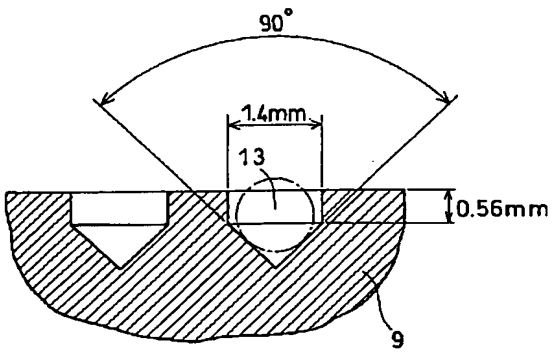
【符号の説明】

1：制御装置、2：制御補助装置、3：モニター、4：導入針移動台、5：導入装置、6：導入針、7：CCDカメラ、8：デジタルカメラ、9：トレイ、10：光源、11：直交移動台、12：水平移動台、13：卵母細胞、14：両生類用生理食塩水、21：容器、22：ラベル、23：保冷剤、31：ヒスタミン受容体遺伝子、32：ヒスタミン受容体、33：ヒスタミンを含んだ試料、34：ヒスタミン応答（あり）、35：ヒスタミンを含まない試料、36：ヒスタミン応答（なし）、41：スポイト、42：両生類用緩衝液、43：抗生物質、44：外容器、45：梱包材

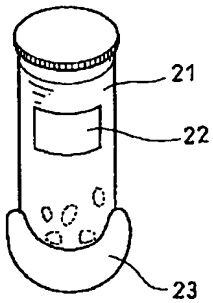
【図１】



【図２】



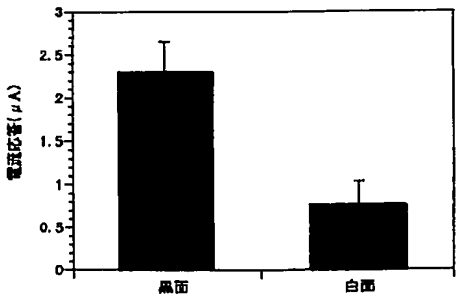
【図３】



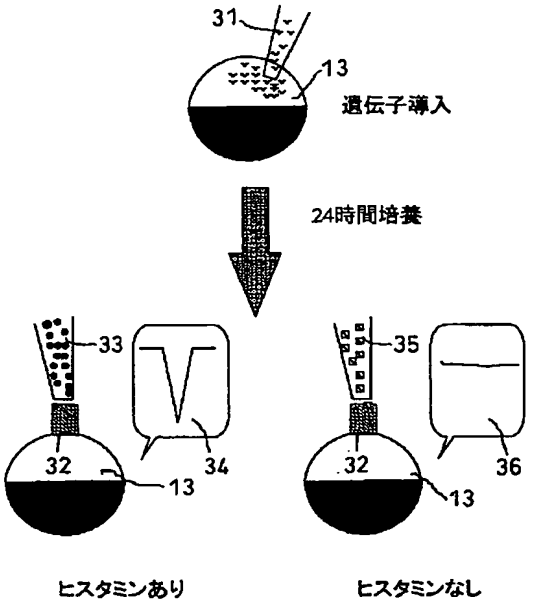
【図４】

	細胞数	反応細胞数
明	40	34
暗	28	1

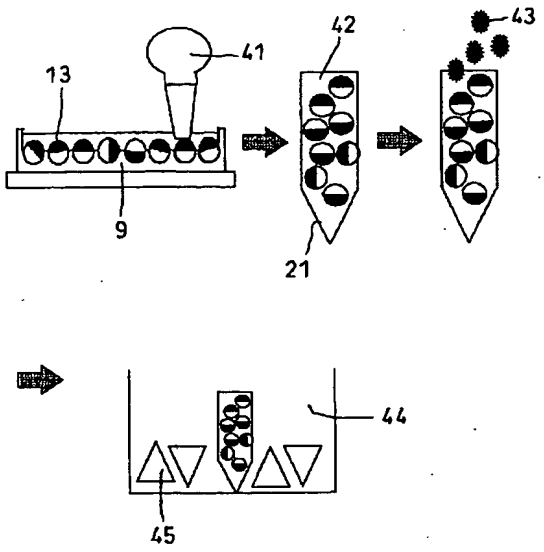
【図５】



【図６】



【図７】



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